

(b) denaturing and renaturing partially purified IGF species;

(c) subjecting renatured IGF species to hydrophobic interaction chromatography; and

C²
(d) performing reverse phase high performance liquid chromatography to obtain a further purified IGF mixture, wherein the further purified IGF mixture has a greater amount of authentic, properly folded IGF than the partially purified IGF mixture, and further wherein only one cation exchange step is performed in the method.

C³
58. (Amended) The method of claim 47, wherein the reverse phase high performance liquid chromatography is performed using a [C₃] C₄ to C₁₀ silica-derivatized resin.

Please ~~cancel~~ claims 2, 6, 13-16, 48, 52 and 59-62, without prejudice and disclaimer.

Remarks

Introductory Comments:

Claims 1-18 and 47-64 were examined in the Office Action dated March 17, 1998 and rejected under (1) 35 USC §112, first paragraph (claim 58); (2) 35 USC §102(e), as anticipated by U.S. Patent No. 5,650,496, to Brierley et al. ("Brierley") (claims 1, 2, 5-11, 13, 14, 17, 47, 48, 51-57, 59-60, 63 and 64); and (3) 35 USC §103, as obvious over Brierley in view of Holtz et al. (the particular Holtz reference relied upon was not specified in the Office Action) and International Publication No. WO 96/07744 to Bussineau et al. ("Bussineau"). These rejections are believed to be overcome in part by the above amendments and are otherwise traversed for the reasons discussed below.

Applicants note with appreciation the withdrawal of the previous rejections under 35 USC §102 and §103, over U.S. Patent No. 5,446,024, to Builder et al., as these rejections were not reiterated.

Overview of the Above Amendments:

The claims have been amended to recite the subject invention with greater particularity. Specifically, claims 1 and 47 now recite a method for "isolating" an authentic, properly folded IGF polypeptide from a "medium into which an IGF polypeptide has been secreted by *Pichia pastoris* cells expressing the IGF polypeptide." Further, the method uses only one cation exchange step.

Claim 58 now recites that the reverse phase high performance liquid chromatography is performed using a C₄ to C₁₀ silica-derivatized resin.

Support for the amendments can be found throughout the specification at, e.g., page 8, lines 9-10; page 20, lines 1-2; page 20, line 16; and page 30, lines 27-19. Thus, no new matter has been added to the application by way of the foregoing amendments.

Claims 2-4, 6, 13-16, 48-50, 52 and 59-62 have been cancelled. Cancellation of the claims is without prejudice, without intent to abandon any originally claimed subject matter, and without intent to acquiesce in any rejection of record. Applicants expressly reserve the right to bring the claims again in a related application.

Rejections Over the Art:

Claims 1-2, 5-11, 13, 14, 17, 47, 48, 51-57, 59, 60, 63 and 64 were rejected under 35 USC §102(e), as anticipated by Brierley. The Action states:

The reference teaches a method of purification of IGF that comprises a cation exchange step (sufylpropylated matrix), followed by refolding of the protein with a buffer, followed by hydrophobic-interaction chromatography (butyl substituted polymethacrylate matrix), followed by a second cation exchange step (sufylpropylated matrix) and finally purified by reverse phase chromatography....

Office Action, page 3. However, applicants do not agree that their claimed method is anticipated by Brierley.

In particular, the methods recited in independent claims 1 and 47 utilize preparative reverse-phase high performance (high pressure) liquid chromatography (HPLC) following the hydrophobic interaction chromatography step. Brierley, on the hand, does not describe the use of preparative reverse phase HPLC, but rather uses a low performance (low pressure) packing (Amberchrome CG1000sd™) in the reverse phase step. Further, Brierley uses two cation exchange chromatography steps. However, the instant method specifically recites the use of a single cation exchange chromatography step. Since Brierley does not teach the use of preparative reverse phase HPLC or a method with only one cation exchange step, Brierley does not anticipate the present claims. Hence this basis for rejection should be withdrawn.

The claims were also rejected under 35 USC §103(a) as unpatentable over Brierley in view of Holtz and Bussineau. The Office applies Brierley as above and asserts:

[T]he difference between the prior art and the instant application is that the reference does not teach the use of yeast cell, *S. cerevisiae*, for the recombinant production of IGF and the purification of IGF II.

However, Bussineau et al. teach a method of recombinant production of IGF utilizing *S.*

cerevisiae (see example 1). The reference further teaches that at the end of the fermentation period, an alkaline shock treatment, wherein an alkali is added to adjust the final pH of the culture medium to the range of 8-11, is conducted...Therefore, it would have been obvious to one of ordinary skill in the art to use *S. cerevisiae* as the yeast cells and further to use an alkaline shock treatment, as outlined by Bussineau et al., to obtain a higher yield in protein.

Office Action, page 4. However, applicants submit that the combination is wholly improper and that this basis for rejection should be withdrawn.

In particular, the Office has failed to identify which Holtz reference is being used in the combination. Applicants note that at least three Holtz references have been cited in the various Information Disclosure statements provided to the Office and that several other patents to Holtz exist. Not only has the Office failed to properly identify the Holtz reference used in the combination, the Office has also failed to explain the basis for its reliance on Holtz. Thus, applicants cannot even begin to address the rejection. Accordingly, the Office has failed to present a *prima facie* case of obviousness and the cited combination must fail. The Office is reminded that should this combination be reiterated in a subsequent Office Action, the Action cannot properly be made final.

Nevertheless, applicants will address the Office's statements with respect to Brierley and Bussineau. Section 2142 of the MPEP sets forth the following basic requirements for *prima facie* obviousness: (1) there must be some suggestion or motivation to modify the references or combine reference teachings; (2) there must be a reasonable expectation of success (for the modification); and (3) the combination must teach or suggest all of the claim

limitations. Furthermore, the teaching or suggestion and the reasonable expectation of success must both be found in the prior art, not in applicants' disclosure. Applicants submit that the Office has failed to satisfy each of these criteria and has thus failed to establish *prima facie* obviousness.

As explained above, Brierley does not teach or suggest a method of isolating an IGF polypeptide from a medium into which the polypeptide has been secreted by *Pichia pastoris* cells expressing the same, wherein the method uses reverse phase HPLC and a single cation exchange column. In fact, applicants achieve at least comparable, if not better purity, despite the fact that a single cation exchange step is used. See, e.g., Examples IIA and IIB. Applicants submit that a modification of Brierley to result in such a method is not suggested by the cited art.

As correctly noted by the Office, Bussineau pertains to methods for improved production of IGF. One method described in Bussineau, "alkaline shock treatment," entails elevating the pH of a fermentor culture at the completion of fermentation and prior to cell removal. See, e.g., page 8, lines 3-5 of Bussineau. Bussineau applies this method to cultures of *Saccharomyces cerevisiae* cells, not *Pichia* cells. At best, then, the combination of Brierley with Bussineau might lead one of skill in the art to incorporate an alkaline shock step into an IGF purification method as taught by Brierley, from *S. cerevisiae* cells prior to the first cation exchange step. However, the combination certainly does not teach substituting a preparative reverse phase HPLC step for a reverse phase low performance step and does not suggest the use of a purification method with only one cation exchange step, for purification of IGF from *P. pastoris* medium.

There is no suggestion to modify Brierley's method to that of applicants' and no indication in Brierley or Bussineau that applicants' methods would be successful for purifying an authentic, properly folded IGF polypeptide. Without a suggestion to modify the reference evident in the prior art, the only conclusion supported by the record is that the rejection was made impermissibly using hindsight reconstruction of the invention. As stated by the Court of Appeals for the Federal Circuit, "[i]t is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992). See, also, *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988): "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention."

Thus, applicants submit that the claimed invention is nonobvious over the art and request reconsideration and withdrawal of this ground of rejection.

Conclusion

Applicants respectfully submit that the pending claims define an invention which is novel and nonobvious over the art. Accordingly, allowance is believed to be in order and an early notification to that effect would be appreciated.

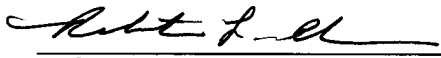
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Claims (08/477,984)

1. (Amended) A method for isolating an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a medium into which the IGF polypeptide has been secreted by *Pichia pastoris* cells expressing the IGF polypeptide, wherein the method comprises:

(a) performing a cation exchange chromatography with the medium to yield a first IGF mixture;

(b) denaturing and renaturing IGF species present in the first IGF mixture to yield a second IGF mixture;

(c) subjecting the second IGF mixture to hydrophobic interaction chromatography to yield a third IGF mixture; and

(d) performing reverse phase high performance liquid chromatography on the third IGF mixture to yield a fourth IGF mixture, wherein the fourth IGF mixture has a greater amount of authentic, properly folded IGF than the first IGF mixture, and further wherein only one cation exchange step is performed in the method.

3. The method of claim 1, wherein the method further comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 8 to about pH 12, prior to the first cation exchange chromatography.

4. The method of claim 3, wherein the method comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 10 to about pH 11, prior to the first cation exchange chromatography.

5. The method of claim 1, wherein the first cation exchange chromatography is performed using a sulfopropylated matrix.

7. The method of claim 1, wherein the denaturing and renaturing steps are performed together using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in

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sufficient amounts and under conditions which allow for the reduction and subsequent oxidation of disulfide bonds.

8. The method of claim 7, wherein the denaturation buffer comprises about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

9. The method of claim 8, wherein the denaturation buffer comprises about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

10. The method of claim 1, wherein the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

11. The method of claim 1, wherein the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

12. The method of claim 1, wherein the reverse phase high performance liquid chromatography is performed using a C₈ silica-derivatized resin.

17. The method of claim 1, wherein the IGF is IGF-I.

18. The method of claim 1, wherein the IGF is IGF-II.

47. (Amended) A method for isolating an authentic, properly folded insulin-like growth factor (IGF) polypeptide from a medium into which the IGF polypeptide has been secreted by

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Pichia pastoris cells expressing the IGF polypeptide, wherein the method comprises:

- (a) performing a cation exchange chromatography with the [yeast cell] medium to obtain a partially purified IGF mixture;
- (b) denaturing and renaturing partially purified IGF species;
- (c) subjecting renatured IGF species to hydrophobic interaction chromatography; and
- (d) performing reverse phase high performance liquid chromatography to obtain a further purified IGF mixture, wherein the further purified IGF mixture has a greater amount of authentic, properly folded IGF than the partially purified IGF mixture, and further wherein only one cation exchange step is performed in the method.

49. The method of claim 47, wherein the method further comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 8 to about pH 12, prior to the first cation exchange chromatography.

50. The method of claim 49, wherein the method comprises raising the pH of the yeast cell medium which comprises yeast cells to about pH 10 to about pH 11, prior to the first cation exchange chromatography.

51. The method of claim 47, wherein the first cation exchange chromatography is performed using a sulfopropylated matrix.

53. The method of claim 47, wherein the denaturing and renaturing steps are performed together using a denaturation buffer comprising urea, dithiothreitol, alcohol and salt, in sufficient amounts and under conditions which allow for the reduction and subsequent oxidation of disulfide bonds.

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54. The method of claim 53, wherein the denaturation buffer comprises about 1 to about 4 M urea, about 1 mM to about 75 mM sodium borate, about .5 M to about 3 M sodium chloride, about 10% to about 30% ethanol and about 0.5- to about 7-fold molar excess of dithiothreitol.

55. The method of claim 54, wherein the denaturation buffer comprises about 1.5 M to about 3 M urea, about 3 to about 50 mM sodium borate, about 1 M to about 1.5 M sodium chloride, about 15% to about 25% ethanol, and about an equimolar to about a 5-fold molar excess of dithiothreitol.

56. The method of claim 47, wherein the hydrophobic interaction chromatography is performed using a polyethyleneamine matrix.

57. The method of claim 8, wherein the hydrophobic interaction chromatography is performed using a butyl- or phenyl-substituted poly(methacrylate) matrix.

58. (Amended) The method of claim 47, wherein the reverse phase high performance liquid chromatography is performed using a C₄ to C₁₀ silica-derivatized resin.

63. The method of claim 47, wherein the IGF is IGF-I or an analog thereof.

64. The method of claim 63, wherein the IGF is IGF-I.